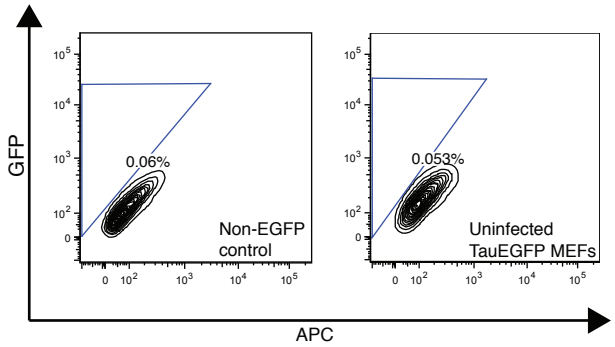
a

Protein stained	TauEGFP MEFs	Balb/c MEFs	TauEGFP TTFs
Tuj1	<0.1%*	<0.1%*	<0.1*
Sox2	Absent	Absent	Absent
Brn2	Absent	Absent	Absent
GFAP	Absent	Absent	Absent
P75	Absent	Absent	Absent
Cytokeratins	<0.1%	n.d.	n.d.
Pax3	<0.1%	n.d.	<0.1%
Pax6	Absent	n.d.	Absent
Pax7	Absent	n.d.	Absent
Nkx2.2	Absent	n.d.	Absent
Olig1	Absent	n.d.	Absent

*Cells with a fibroblast-like morphology

b

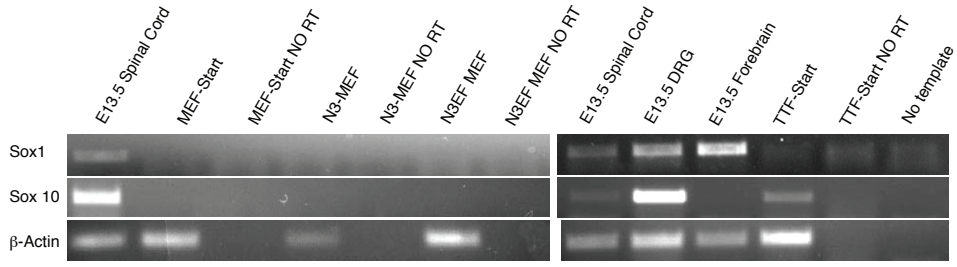


c

	TauEGFP MEFs			TauEGFP perinatal TTF		
	Neural media	Neural media+EGF +FGF2	Neural media (GF withdrawal)	Neural media	Neural media+EGF +FGF2	Neural media (GF withdrawal)
Sox2	Absent	Absent	Absent	Absent	Absent	Absent
Brn2	Absent	Absent	Absent	Absent	Absent	Absent
GFAP	Absent	<0.1%	Absent	Absent	Absent	Absent
MAP2	Absent	Absent	Absent	Absent	Absent	Absent
Tuj1	<1%*	<1%*	<1%*	<1%*	<1%*	<1%*
TauEGFP	Absent	Absent	Absent	Absent	Absent	Absent

*Cells with a fibroblast-like morphology

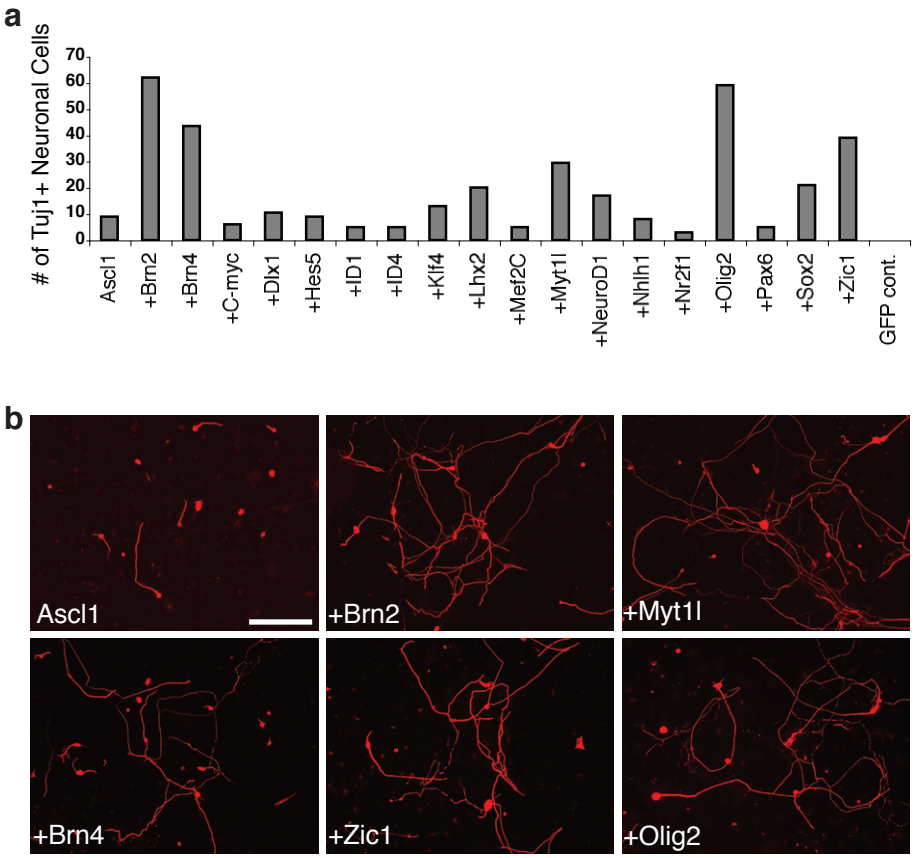
d



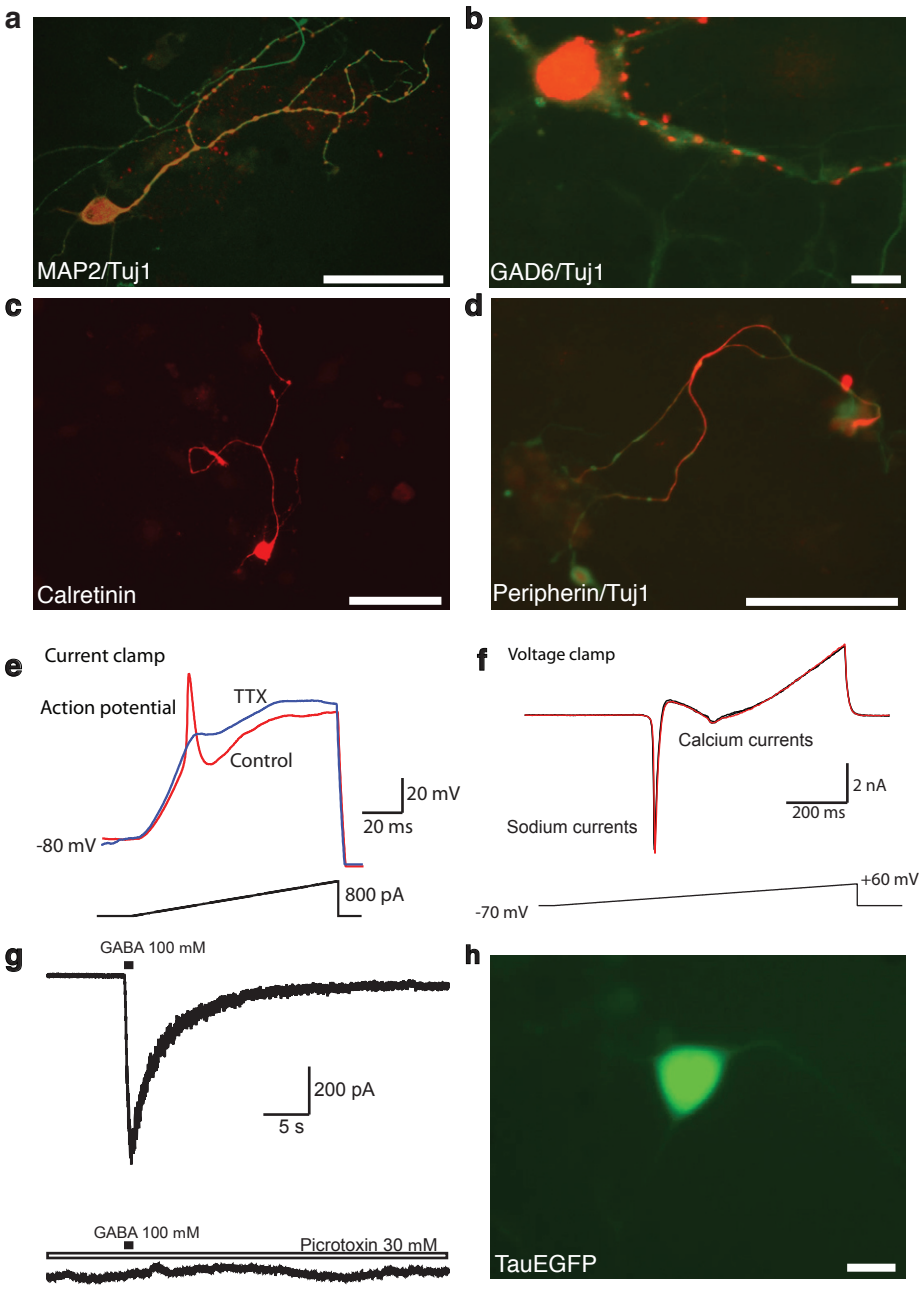
Supplementary Figure 1: Characterization of MEF and tail-tip fibroblast cultures

a, Passage 3 TauEGFP MEF, Balb/c MEF, and TauEGFP TTF cultures were immunostained with antibodies against the listed antigens. Each antibody was independently validated using an appropriate positive control. The listed of antigens includes multiple markers for neural stem cells (Sox2, Brn2, GFAP), peripheral and spinal neural progenitor cells (p75, Pax3, Pax6, Pax7, Nkx2-2, Olig1) and markers for neurons and astroglia (Tuj1, TauEGFP, GFAP, Olig1).

Listed percentages are out of >4500 cells. Absent means no positive cells were detected in the stained field. n.d. means fibroblast cultures were not stained. **b**, FACs analysis of uninfected P3 TauEGFP MEFs and control BALB/c MEFs for GFP fluorescence. Graph plots GFP fluorescence (y-axis) against APC (x-axis). **c**, Characterization of passage 3 TauEGFP MEFs and perinatal TTFs after culturing in neural media. Cells were either cultured in N3 media for 12 days (to promote the differentiation of potentially contaminating neural progenitor cells), N3 media with EGF and FGF2 for 12 days (a condition promoting neural progenitor cell expansion), or N3 with EGF and FGF2 for 8 days followed by growth factor withdrawal for 5 days (to first expand and then differentiate any potentially existing neural progenitor cells). Under no conditions could we detect the presence of neural cell types, only in one condition rare cells were labeled above background with a polyclonal antibody against GFAP. At least 10,000 cells were screened for each staining. **d**, Reverse transcription-PCR on cDNA isolated from passage 3 TauEGFP MEF and Rosa-rtTA TTF cultures. Sox1 and Sox10 could not be detected in MEFs grown in MEF media (MEF-Start), MEFs grown in N3 media (N3-MEF) for 8 days, or in MEFs grown in N3 with EGF and FGF2 for 8 days (N3EF-MEF). TTFs appear to express Sox10 at a low level. Positive controls included E13.5 spinal cord, E13.5 dorsal root ganglia (DRG), and E13.5 forebrain cDNAs. For each experimental sample a control reaction was carried without reverse transcriptase (No RT).

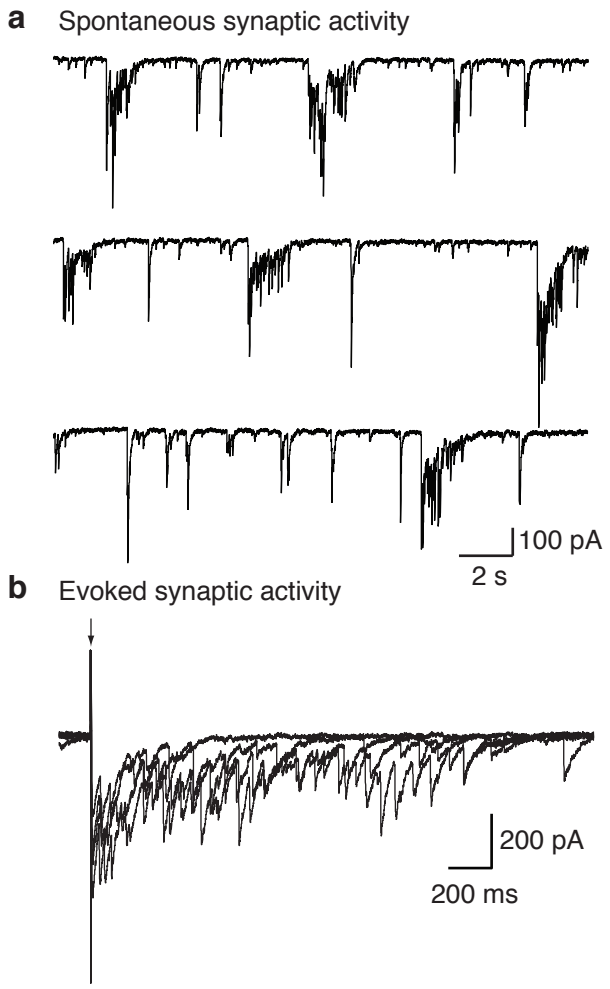


Supplementary Figure 2: Screen for enhancers of Ascl1-induced conversion
a, The effect of 18 transcription factors in combination with Ascl1 on neuronal induction 13 days post infection. Shown are the average numbers of Tuj1-positive cells with a process three times longer than the cell body derived from two randomly selected, low magnification visual fields. **b**, Representative Tuj1-positive cells 13 days after infection with Ascl1 alone or in combination with the indicated genes. Note the increased complexity of the neurites in the Ascl1+Myt1l condition.



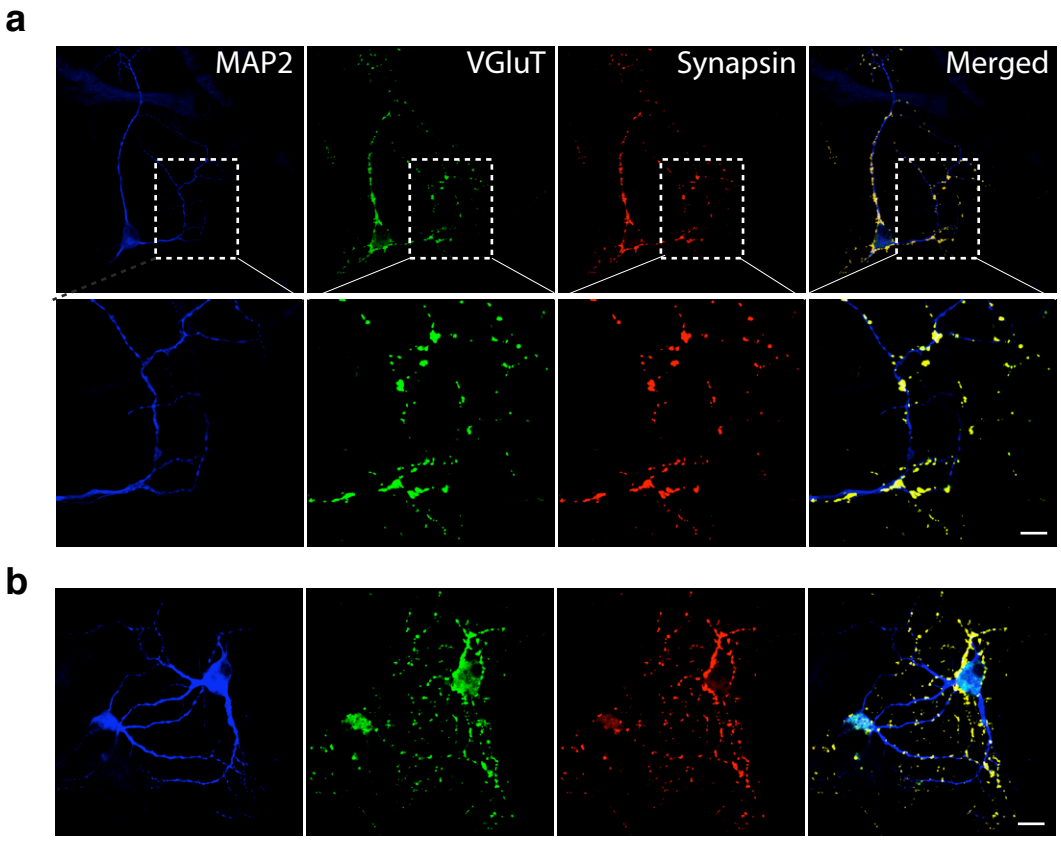
Supplementary Figure 3: Further immunohistochemical and electrophysiological characterization of 5F-iN cells

a, iN cells derived from Balb/c MEFs stained for MAP2 (red) and Tuj1(green). **b,c,** At day 22 post-infection TauEGFP MEF-derived 5F iN cells rarely express GAD6. (b) Calretinin (red,c) and Tuj1 (green,c). **d**, An iN cell derived from Rosa26-rtTA TTFs that expressed the peripheral neuron marker peripherin (red) and Tuj1 (green). **e**, Representative traces of an action potential (AP) elicited using a ramp protocol (insert) from a TauEGFP MEF-derived iN cell at 8 days post infection. AP was abolished after application of TTX (both traces are from the same cell). **f**, Superimposed whole cell currents recorded by using a ramp protocol (insert) revealing fast-inactivating sodium current and inward calcium currents. **g**, TauEGFP MEF-derived iN cells respond to exogenous application of 100 μM GABA through a picosprizer. Lower panel showing that the GABA induced current response could be blocked by application of 30 μM picrotoxin. **h**, TauEGFP-expressing 5F iN cell observed in a MEF culture 5 days post infection. Scale bars = 10 μm (b,h) and 100 μm (a,c,d).

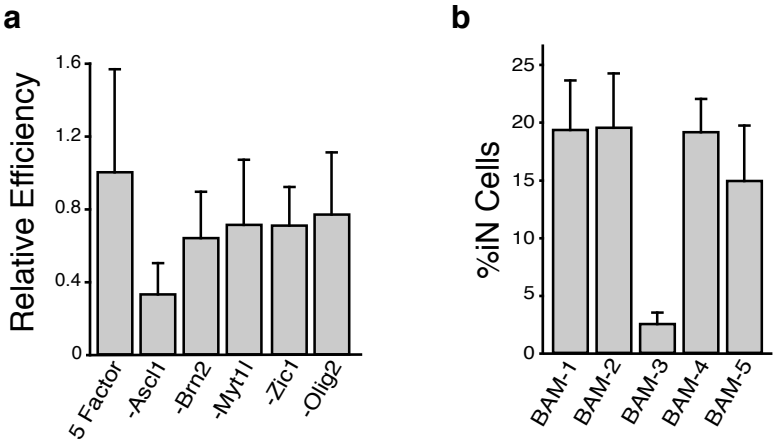


Supplementary Figure 4: Synaptic integration of tail tip fibroblast-derived 5F-iN cells in cortical neural networks

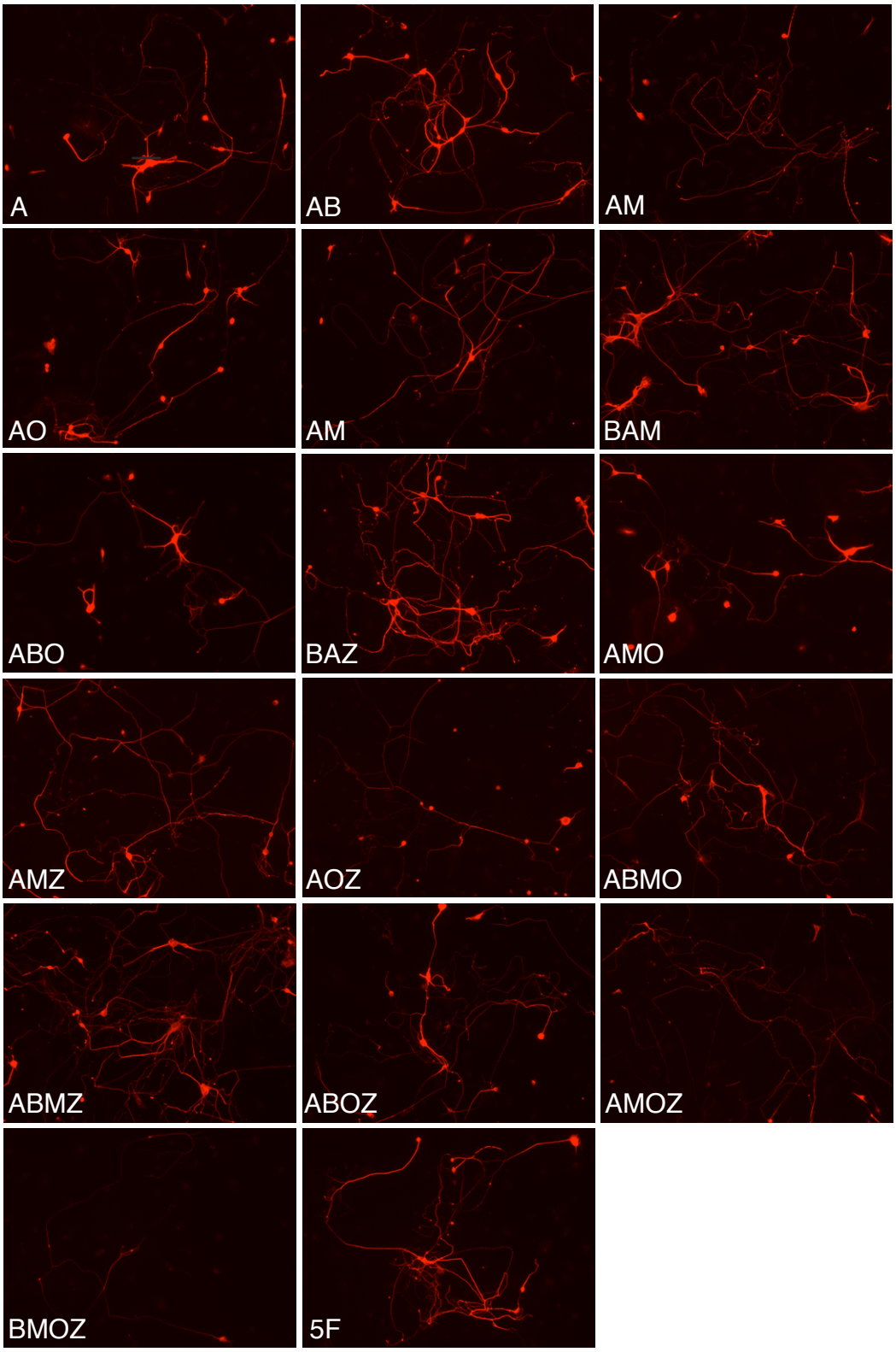
5F perinatal tail fibroblast-iN cells were FACS-sorted for EGFP expression 7-8 days post infection and plated on cortical neuronal cultures (7 days in vitro). Electrophysiological recordings from the TauEGFP cells were performed 7 days after sorting. **a**, Representative consecutive traces of spontaneous synaptic network activities recorded from a TTF-iN cell. **b**, Representative evoked synaptic activity following stimulation (indicated by arrow). Four superimposed responses are shown.



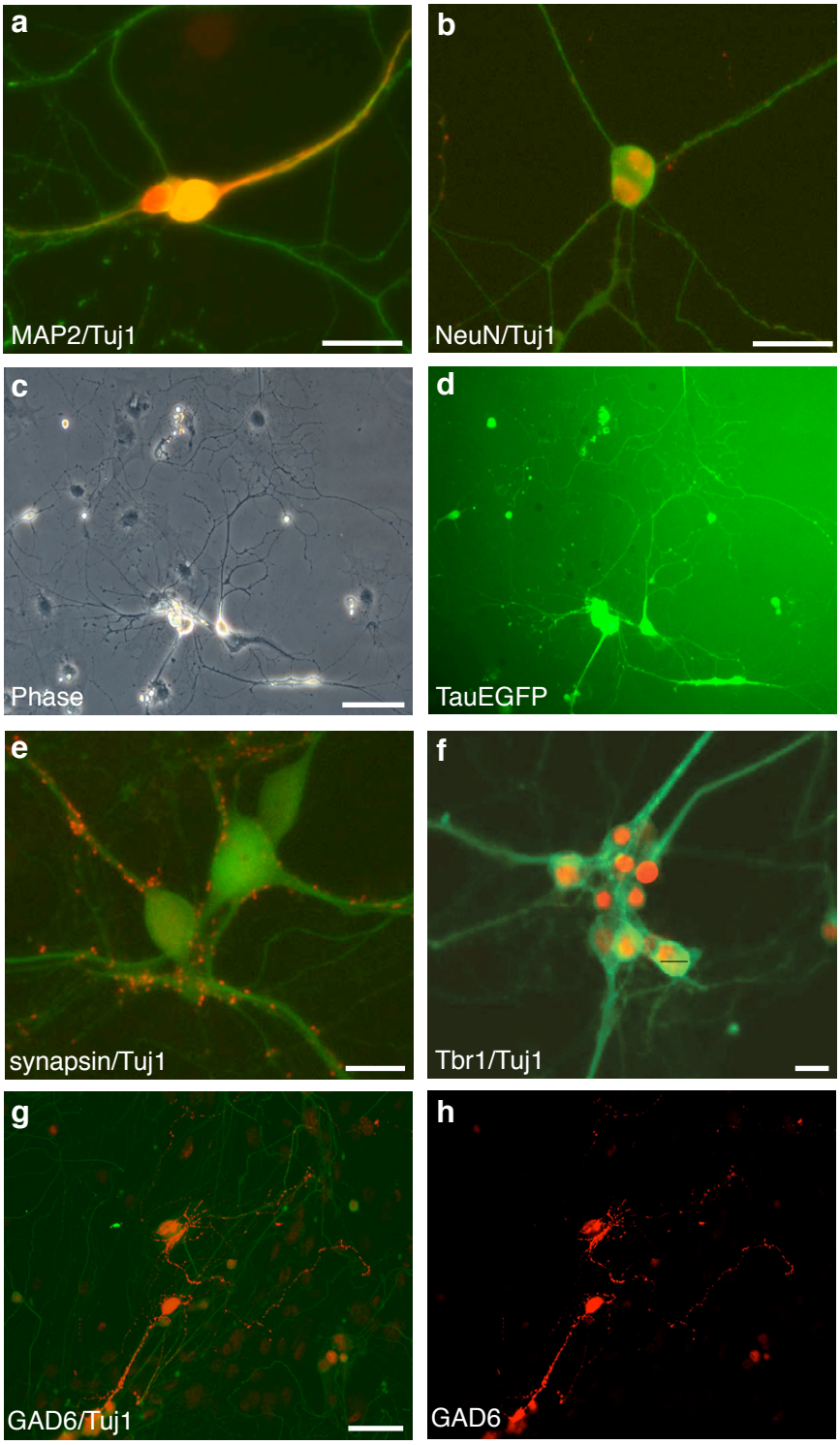
Supplementary Figure 5: Immunofluorescence of 5F-iN cells co-cultured with glial cells
a-b, MEF-derived 5F-iN cells on glia express markers of glutamatergic neurons. Immunostaining for vGLUT1, MAP2, and synapsin. The second row in b is a close-up of the outlined region in the first row. Scale Bars= 10 μm (upper panel a, b), 3 μm (lower panel, a).



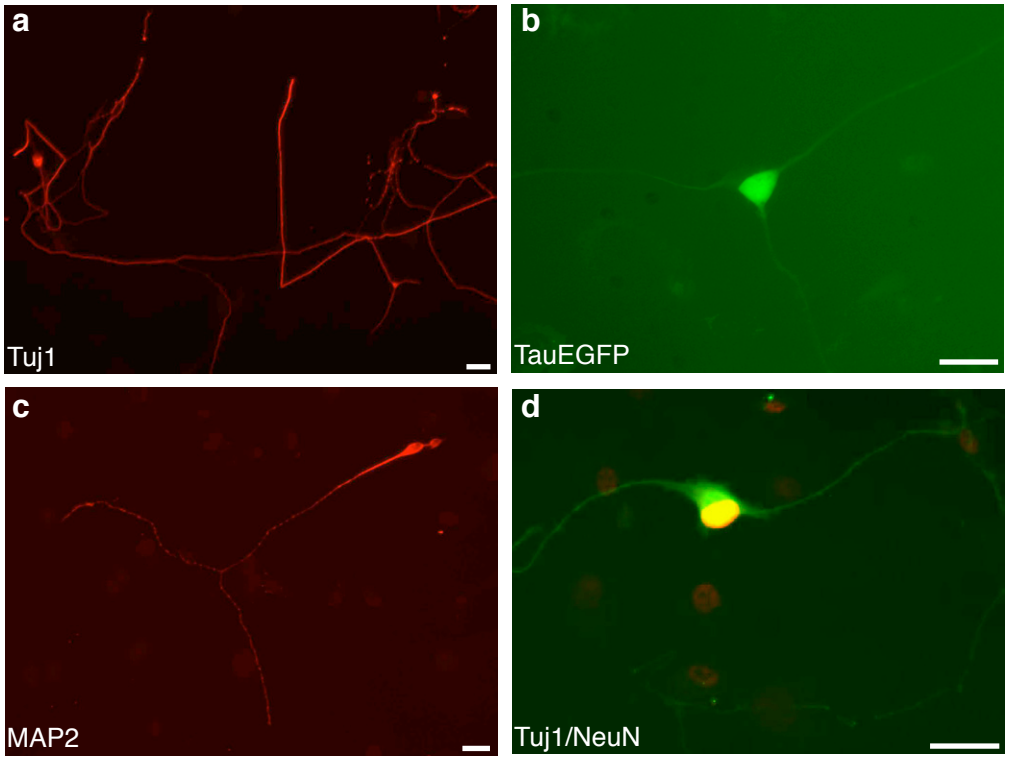
Supplementary Figure 6: Additional neuronal induction efficiency estimates
a, Effect of removing single genes from the 5F pool. The average number of Tuj1-positive neuronal cells visible in a 20x field is normalized to the 5F condition (n=30 visual fields). **b**, Reproducibility of BAM-iN cell generation. Each bar represents an independent experiment. %iN cells is calculated from the number of plated cells (see methods). The low efficiency in BAM-3 is likely due to suboptimal lentiviral titer, however, the iN cells that are present in this condition still exhibit mature neuronal morphologies. Error bars = S.D.



Supplementary Figure 7: Representative images from 1 to 5 factor infections
Tuj1 stainings of iN cells induced by the indicated 1 to 5 factor combinations of the genes Ascl1 (A), Brn2 (B), Myt1L (M), Olig2 (O) and Zic1 (Z) 12 days after infection. Total virus is kept constant between different factor combinations. Scale bar = 50 μm.



Supplementary Figure 8: Additional characterization of BAM-iN cells
a-b, Day 12 TTF-derived BAM iN cells express the pan-neuronal markers MAP2 (a, red) and NeuN (b, red) **c-d**, Day 21 TTF-derived BAM iN cells exhibit mature neuronal morphologies and express TauEGFP. **e**, Day 21 TTF-derived BAM-iN cells exhibit punctate synapsin staining. **f**, MEF-derived BAM iN cells express Tbr1, a marker of cortical neurons 22 days after infection. **g-h**, A MEF-derived BAM-iN cell expressing GAD6 (g, red, h) and Tuj1 (g, green). Scale bars = 20 μm (a,b), 50 μm (c,g), 10 μm (e,f).



Supplementary Figure 9: BAM-iN cells derived from adult TTF
BAM iN cells derived from TTF isolated from a six-week-old TauEGFP mouse express Tuj1 (**a, d**, green), TauEGFP (**b**), MAP2 (**c**) and NeuN (**d**, red). Scale bars = 20 μm (a-d).

Supplemental Table 1: Transcription factors screened for neuron-inducing activity in MEFs

Gene Name	Gene Bank
Ascl1	NM_008553
Brn2	NM_008899
Brn4	NM_008901
c-myc	NM_010849
Dlx1	NM_010053
Hes5	NM_010419
Id1	NM_010495
Id4	NM_031166
Klf4	NM_010637
Lhx2	NM_010710
Mef2c	NM_025282
Myt1l	NM_001093775
NeuroD1	NM_010894
Nhlh1	NM_010916
Nr2f1	NM_010151
Olig2	NM_016967
Pax6	NM_013627
Sox2	NM_011443
Zic 1	NM_009573

Supplemental Table 2: Numeric electrophysiological parameters recorded from iN cells in different conditions

Electrophysiological parameters of iN cells

MEF-derived 5F-iN cells: Passive membrane properties

Resting Membrane Potentials (mV)

	Average	SEM	n	P value (student *t* test)
Day 8	-30.8	3.2	16	D8 vs. D12 0.000166; D7 Vs. D20:0.00004
Day 12	-47.7	2.8	18	D12 vs. D21: 0.0833
Day 20	-55.4	5.3	12	

Membrane Input Resistance (GΩ)

	Average	SEM	n	P value (student *t* test)
Day 8	1.40	0.20	17	D8 vs. D12: 0.000638; D8 vs. D20: 0.001962
Day 12	0.60	0.10	21	D12 vs. D20: 0.343235
Day 20	0.55	0.07	14	

Membrene Capacitance (pF)

	Average	SEM	n	P value (student *t* test)
Day 8	27.9	2.8	18	D8 vs. D12: 0.774473; D8 vs. D20: 0.020507
Day 12	28.9	2.5	21	D12 vs. D20: 0.016909
Day 20	44.6	6.9	14	

MEF-derived 5F-iN cells: Active membrane properties

Spontaneous action potential firing and induced Action potentials (AP)

	spontaneous	induced	No. of total recordings
Day 8	1	14	17
Day 12	3	14	18
Day 20	2	12	7 (spontaneous), 12 (induced)

AP height (mV)

	Average	SEM	n	P value (student *t* test)
Day 8	84.5	4.5	6	D8 vs. D12: 0.5612; D8 vs. D20: 0.03558
Day 12	81.3	3.0	14	D12 vs. D20: 0.001843
Day 20	94.9	2.3	12	

Note: AP height was measured from baseline. APs were analyzed when first appeard during step depolarizations. 7 cells at D8 are not included due to different protocol used; 1 cell at 7 days has clear AP but with distorted shape and thus not included

AP threshold (mV)

	Average	SEM	n	P value (student *t* test)
Day 8	-25.2	1.5	6	D8 vs. D12: 0.093417; D8 vs. D20: 0.031866
Day 12	-29.0	1.3	14	D12 vs. D20: 0.436939
Day 20	-30.5	1.4	12	

Note: AP threshold was measured from the beginning of the upstroke of the action potential

Maximal sodium current (nA)

	Average	SEM	n	P value (student *t* test)
Day 8	700.2	257.2	5	D8 vs. D12: 0.534945; D8 vs. D20: 0.091192
Day 12	532.7	105.2	6	D12 vs. D20: 0.050369
Day 20	3615.0	1287.6	7	

Note: Maximal sodium currents were measured at voltage clamp mode using step depolarization proto col

TTF-derived 5F-iN cells: Passive and active membrane properties on Day 12

	Average	SEM	n
Resting membrane potential (mV)	-57.2	7.2	11
Membrane input resistance (GΩ)	0.3	0.0	11
Membrane Capacitance (pF)	26.1	1.4	11
AP	Observed in 9 out of 11 cells, 2 of them fire repetitively		

MEF-derived BAM-iN cells: Passive and active membrane properties (co-cultured with glia)

	Average	SEM	n
Membrane input resistance (GΩ)	0.9	0.1	18
Membrane Capacitance (pF)	33.9	3.9	18
AP	Not assayed due to use CsCl internal solutions.		

TTF-derived BAM-iN cells: Passive and active membrane properties (co-cultured with glia)

	Average	SEM	n
Membrane input resistance (GΩ)	0.5	0.1	12
Membrane Capacitance (pF)	35.4	5.3	12
AP	Not assayed due to use CsCl internal solutions.		

MEF-derived 5F-iN cells: Synaptic functions (co-cultured with glia)

		Average	SEM	n	No. of total recordings
AMPA EPSCS		229.6	75.2	8	11
NMDA EPSCS		743.9	224.8	9	11
Spontaneous PSCs	observed in 5 cells				11
IPSCs	No obvious events observed, 11 recordings without blockers, 4 with APV+CNQX				

Note: AMPA EPSCs were recorded a t Vh of -70 mV; NMDA EPSCs were recorded at +60 mV and measured at 50 ms after stimulation.
Spontaneous PSCs were recorded in the absence of blockers.

MEF-derived 5F-iN cells: Synaptic integrations (co-cultured with cortical neurons)

	Average	SEM	n	No. of total recordings
IPSC Amplitude (pA)	1228.2	275.1	13	15
AMPA EPSC Amplitude (pA)	94.7	21.5	9	9
NMDA EPSC Amplitude (pA)	280.4	89.4	9	9
Spontaneous PSCs	observed in 6 cells			6
Evoked PSCs	compond PSCs observed in 6 cells			6
Spontaneous IPSCs	observed in 13 cells			15
Spontaneous EPSCS	observed in 4 cells			9

Note: PSCs were recorded without blockers; IPSC: in APV and CNQX; EPSC: in picrotoxin

TTF-derived 5F-iN cells: Synaptic integrations (co-cultured with cortical neurons)

Spontaneous PSCs	observed in 2 out of 3 cells recorded
Evoked PSCs	compond evoked PSCs observed in 2 out of 3 cells

Note: No blockers were added for these recordings.

MEF-derived BAM-iN cells: Synaptic Function (co-cultured with glia)

	Average	SEM	n	No. of total recordings
AMPA EPSC Amplitude (pA)	41.7	11.9	9	16
NMDA EPSC Amplitude (pA)	130	33.8	11	16
Spontaneous PSCs	observed in 3 cells			16
IPSC Amplitude (pA)	not detected			16

Note: AMPA EPSCs were recorded at Vh of -70 mV; NMDA EPSCs were recorded at +60 mV and measured at 50 ms after stimulation

TTF-derived BAM-iN cells: Synaptic Function (co-cultured with glia)

	Average	SEM	n	No. of total recordings
AMPA EPSC Amplitude (pA)	82.8	27.3	5	12
NMDA EPSC Amplitude (pA)	208.7	75.0	6	12
Spontaneous PSCs	observed in 3 cells			12
IPSC Amplitude (pA)	not detected			12

Note: AMPA EPSCs were recorded at Vh of -70 mV; NMDA EPSCs were recorded at +60 mV and measured at 50 ms after stimulation

Membrane properties of MEFs infected with Ascl 1, Brn2, Myt1l :Day 12

	Average	SEM	n	P value (student *t* test)
Ascl1	-48.6	3.4	11	A vs AB: 0.729261; A vs AM:0.318123 A vs. 3F:0.378688
Ascl1+Brn2	-47.3	1.8	12	AB vs. AM: 0.08692; AB vs. 3F: 0.125888
Ascl1+Myt1l	-52.9	2.6	12	AM vs. 3F: 0.885015
BAM	-52.4	2.6	13	

Membrane Input Resistance (GΩ)

	Average	SEM	n	P value (student *t* test)
Ascl1	0.95	0.14	11	A vs AB: 0.192394; A vs AM:0.074357; A vs. 3F:0.951576
Ascl1+Brn2	1.31	0.22	12	AB vs. AM: 0.010736; AB vs. 3F: 0.144018
Ascl1+Myt1l	0.64	0.09	12	AM vs. 3F: 0.019898
BAM	0.96	0.09	13	

Membrene Capacitance (pF)

	Average	SEM	n	P value (student *t* test)
Ascl1	18.7	1.1	11	A vs AB: 0.60362; A vs AM:0.243535; A vs. ABM:0.01028
Ascl1+Brn2	19.8	1.7	12	AB vs. AM: 0.534332; AB vs. ABM: 0.025714
Ascl1+Myt1l	21.5	2.0	12	AM vs. ABM: 0.076441
BAM	28.1	2.9	13	

Spontaneous AP firing and induced AP

	spontaneous	induced	No. of total recordings
Ascl1	0	9	11
Ascl1+Brn2	0	12	12
Ascl1+Myt1l	1	11	12
BAM	3	13	13

Supplemental Table 3: Resting membrane potentials, membrane input resistances and membrane capacities of iN cells

	RMP (mV)	Rm (GΩ)	Cm (pF)
MEF-derived 5F-iN cells: Day 8			
Cell 1	-38	2.80	29
Cell 2	-34	2.10	13
Cell 3	-34	0.46	23
Cell 4	-30	1.10	30
Cell 5	-20		
Cell 6	-20	0.96	32
Cell 7	-20	1.50	28
Cell 8	-16	3.70	24
Cell 9		2.70	46
Cell 10	-45	0.41	39
Cell 11	-30	1.60	22
Cell 12	-50	0.65	17
Cell 13	-33	2.00	12
Cell 14	-45	0.74	31
Cell 15	-28	1.80	29
Cell 16	-16	0.85	25
Cell 17	-35	1.60	26
Cell 18	-40	0.49	47
Cell 19	-16	1.00	33

Note: Cell 1 Rs is 40 Mohm, not included in the quantitations.
Cell5 parameter not recorded fully, also not included in the final analysis

MEF-derived 5F-iN cells: Day 12			
Cell 1	-30	0.42	25
Cell 2	-57	0.45	42
Cell 3	-65	0.38	32
Cell 4	-60	0.74	53
Cell5	-46	0.86	22
Cell 6	-35	0.89	27
Cell 7	-27	1.10	25
Cell 8		1.10	19
Cell 9		0.95	21
Cell 10		0.62	19
Cell 11	-49	0.59	22
Cell 12	-65	0.13	26
Cell 13	-45	0.49	34
Cell 14	-47	0.49	53
Cell 15	-45	0.56	37
Cell 16	-56	0.50	27
Cell 17	-43	0.62	32
Cell 18	-26	0.36	22
Cell 19	-50	0.82	18
Cell 20	-55	0.63	29
Cell 21	-57	0.74	21

	RMP (mV)	Rm (GΩ)	Cm (pF)
MEF-derived 5F-iN cells: Day 20			
Cell 1		0.90	45
Cell 2		0.48	40
Cell 3	-49	0.71	17
Cell 4	-64	0.50	39
Cell 5	-60	0.25	103
Cell 6	-30	0.74	22
Cell 7	-47	0.19	66
Cell 8	-61	0.24	87
Cell 9	-57	0.32	45
Cell 10	-52	0.67	30
Cell 11	-68	0.90	28
Cell 12	-56	0.65	24
Cell 13	-52	0.96	20
Cell 14	-69	0.26	58

TTF-derived 5F-iNs cells: Day 12			
Cell 1	-62	0.27	22
Cell 2	-69	0.24	32
Cell 3	-55	0.42	35
Cell 4	-40	0.00	20
Cell 5	-35	0.21	29
Cell 6	-70	0.39	23
Cell 7	-62	0.16	27
Cell 8	-48	0.14	21
Cell 9	-64	0.24	26
Cell 10	-64	0.49	27
Cell 11	-60	0.31	25

MEF-derived Ascl1-iN cells: Day 12			
Cell 1	-50	0.70	21
Cell 2	-45	0.60	23
Cell 3	-55	0.56	15
Cel l4	-55	0.49	21
Cell 5	-43	1.20	23
Cell 6	-30	2.00	22
Cell 7	-60	0.45	17
Cell 8	-54	1.20	13
Cell 9	-67	1.20	18
Cell 10	-43	1.20	13
Cell 11	-33	0.86	19

*In some cases, different internal solutions were used, which did not allow for the measurement of resting membrane potential (RMP). Rm: membrane input resistance; Cm: membrane capacitance

Supplementary Table 3 continued

	RMP (mV)	Rm (GΩ)	Cm (pF)
MEF-derived Ascl1+Brn2-iN cells: Day 12			
Cell 1	-58	1.20	9
Cell 2	-35	3.30	17
Cell 3	-43	1.80	21
Cell 4	-48	0.42	15
Cell 5	-55	0.95	23
Cell 6	-40	0.43	23
Cell 7	-50	1.20	21
Cell 8	-48	1.60	12
Cell 9	-49	0.96	20
Cell 10	-49	0.90	32
Cell 11	-48	1.80	19
Cell 12	-45	1.20	25
MEF-derived Ascl1+Myt1l-iN cells: Day 12			
Cell1	-49	0.63	15
Cell2	-59	0.47	21
Cell3	-62	0.85	22
Cell4	-57	0.34	21
Cell5	-40	1.30	11
Cell6	-65	0.73	24
Cell7	-64	0.63	13
Cell 8	-54	0.34	30
Cell 9	-41	0.53	25
Cell 10	-43	0.55	21
Cell 11	-47	1.10	18
Cell 12	-54	0.23	35
MEF-derived Ascl1+Brn2+Myt1l-iN cells: Day 12			
Cell1	-42	1.10	18
Cell2	-60	0.62	20
Cell3	-52	0.36	46
Cell4	-61	1.40	21
Cell5	-64	1.20	32
Cell6	-66	1.10	30
Cell7	-57	0.73	49
Cell8	-59	0.51	31
Cell 9	-50	1.10	19
Cell 10	-42	1.00	36
Cell 11	-45	1.00	16
Cell 12	-40	0.96	25
Cell 13	-43	1.40	21